

Study of the immature stages of two species of the biting midge genus *Culicoides* (Diptera: Ceratopogonidae)

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Abstract. Pupa of *Culicoides insignis* Lutz, 1913, and larva and pupa of *Culicoides bambusicola* Lutz, 1913 are fully described. They are illustrated and photographed using binocular microscope, phase-contrast stereoscope at oil immersion and scanning electronic microscopy, from material collected in Corrientes and Misiones provinces, Argentina. Palatum, maxilla and hypopharynx of the fourth instar larva of *C. bambusicola* as well as the cephalothoracic sensilla and sensillar pattern of the first and fourth abdominal segment of the pupa are described for both species. The immatures of *C. bambusicola* are compared with *Culicoides debilpalpis* Lutz, 1913. Pupa of *C. insignis* is compared with the following related congeners: *C. maruim* Lutz, 1913, *C. charruus* Spinelli & Martinez, 1992, and *C. annetae* Spinelli & Borkent, 2004.

Key words. Diptera, Ceratopogonidae, *Culicoides bambusicola*, *Culicoides insignis*, larva, pupa, redescription, Neotropical Region

Introduction

Biting midges of the genus *Culicoides* Latreille, 1809 are among the most abundant of haematophagous insects and occur throughout most of the inhabited world. Across this broad range they transmit a great number of assorted pathogens of human, and domestic and wild animals. However, they are as vectors of arboviruses, and particularly arboviruses of domestic livestock, that they achieve their prime importance. Some of these species are known or potential vectors of viruses in the Neotropical region, e.g. *C. insignis* Lutz, 1913 and *C. pusillus* Lutz, 1913 for the bluetongue virus of domestic and wild ruminants (MELLOR et al. 2000; BORKENT 2005).

BORKENT & SPINELLI (2007) listed 267 species of *Culicoides* in their catalogue of Neotropical Ceratopogonidae. Subsequently 13 new species have been described (PERRUOLO 2006;

FELIPPE-BAUER et al. 2008a,b, 2009; SPINELLI et al. 2009; FELIPPE-BAUER et al. 2010; TRINDADE & FELIPPE-BAUER 2011a,b). The immature stages of the genus are poorly known; pupae of 30 Neotropical species and larvae of only 14 species have been described.

The immatures of *Culicoides bambusicola* Lutz, 1913 and *C. insignis* have been known since the last century. However, the descriptions of both species are very incomplete due to lack of the morphological diagnostic characters used nowadays and important for their identification such as cephalothoracic and first segment sensilla. The purpose of this paper is to fully describe the immature stages of *C. bambusicola* and pupae of *C. insignis* from material recently collected in Misiones and Corrientes provinces, Argentina and from material deposited in the collection of División Entomología of Museo de La Plata (MLP).

Material and methods

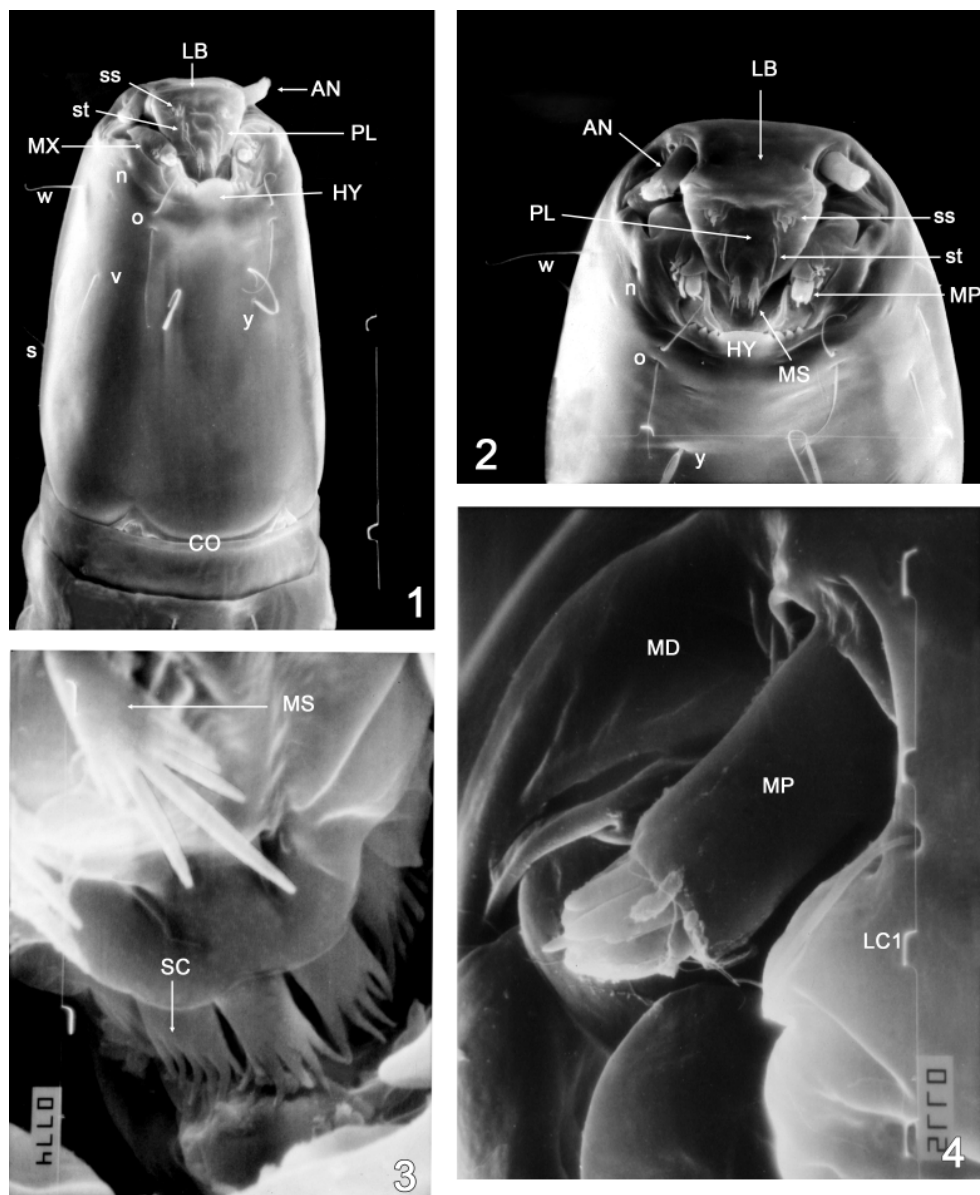
Larvae and pupae of *C. bambusicola* were collected from bamboo canes in Parque Provincial Saltos del Moconá, Misiones and Estación Biológica de Corrientes (EBCo), Corrientes, Argentina. They were taken by making a hole with a drill in the middle of the internodes and extracting the content with a tube connected to a lift pump. Pupae of *C. insignis* were collected from muddy water in the province of Corrientes, in Argentina. They were carried to the laboratory. The larvae were placed individually in Petri dishes and the pupae were conditioned in vials, individually, with a drop of water. Observations were done daily until adult emergence. In order to observe the ultrastructural characters, the immatures were examined using a binocular microscope and a phase-contrast stereoscope in oil immersion. The microphotographs with SEM used in this work were borrowed from RONDEROS & SPINELLI (2000). Measurements and ratios were taken using a binocular microscope (BCM). The adult specimen plus the pupal and larval exuviae were slide-mounted in Canada balsam following the technique described by BORKENT & SPINELLI (2007). The total length of pupae was measured from the front of the head to the apex of the abdomen between the bases of the terminal processes. In pupal exuviae the anterior margin was taken as the anterior margin of the antennal base. Ink illustrations were made with camera lucida. Photographs were taken with a Micrometrics SE Premium digital camera, through a Nikon Eclipse E200 microscope. For terminology of pupae see BORKENT (2012), with addition of the following abbreviations of measurements: DAL, dorsal apotome length, and DAW, dorsal apotome width; for larval structures see RONDEROS et al. (2010). Studied specimens are deposited in the collection of División Entomología, Museo de La Plata, Argentina (MLP).

Results

Culicoides bambusicola Lutz, 1913

(Figs 1–16)

Culicoides bambusicola Lutz, 1913: 62 (female, larva; Brazil); COSTA LIMA (1937: 412) (in key); BARRETTO (1944: 95) (male; Brazil); LANE (1947: 166) (pupa, redescr. larva; Brazil); BARBOSA (1952: 11) (Figs larva, pupa; Brazil); FORATTINI (1957: 321) (redescr. adult, pupa, larva; syn.: *bahiensis*; type locality stated; distr.); WIRTH et al. (1988: 54) (Neotropical atlas, wing); BORKENT & WIRTH (1997: 62) (world catalog); RONDEROS & SPINELLI (2000: 134) (redescr. larva, pupa, Argentina); BORKENT & SPINELLI (2000: 29) (catalog, species south of USA); RONDEROS &



Figs 1–4. *Culicoides bambusicola* Lutz, 1913, larva: 1 – head capsule, ventral view; 2 – head capsule, anteroventral view; 3 – mouthparts (messors and scopae); 4 – head capsule, detail of lateral view of mouthparts (mandible and maxilla) (from RONDEROS & SPINELLI 2000). Abbreviations: AN – antennae; CO – collar; HY – hypostoma; LC1 – lacinial sclerite 1; LB – labrum; MD – mandible; MX – maxilla; MP – maxillary palpus; MS – messors; SC – scopae; PL – palatum; ss – sensilla styloconica; st – sensilla trichoidea. Head capsule chaetotaxy: n – anterolateral pit; o – parhypostomal setae; s – anterior perifrontal setae; v – posterolateral setae; w – anterolateral setae; y – ventral setae.

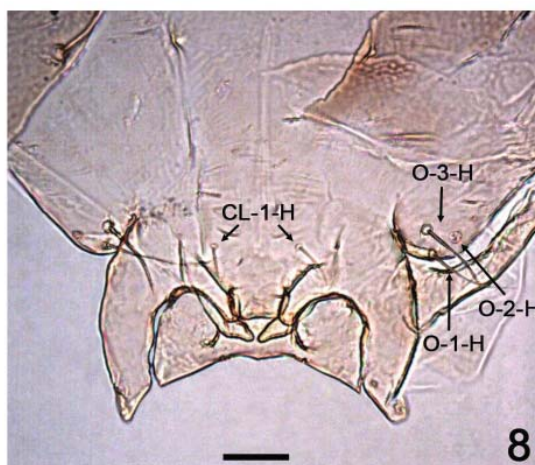
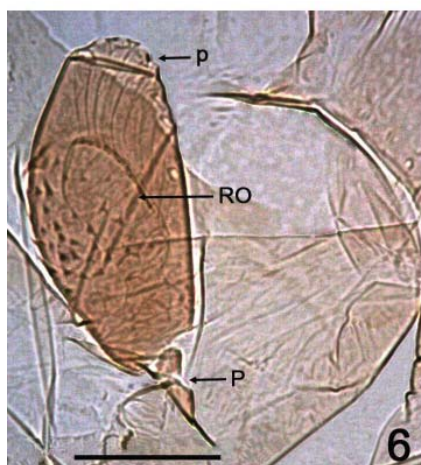
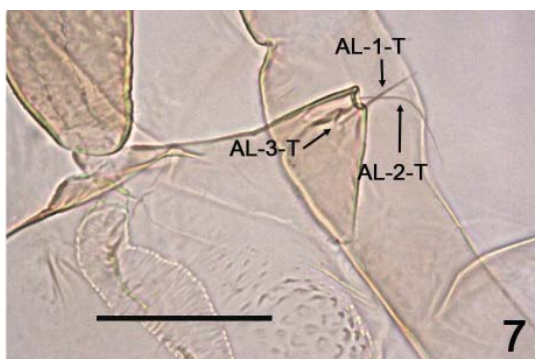
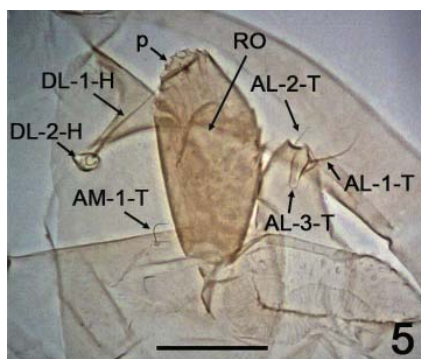
SPINELLI (2002: 93) (Argentina record); RONDEROS & DÍAZ (2002: 44) (rearing; Argentina, Buenos Aires record); SPINELLI et al. (2005: 138, 142) (in key, wing photo; Argentina); BORKENT & SPINELLI (2007: 64) (Neotropical catalog); BORKENT (2013: 75) (online catalog).

Culicoides bahiensis Barbosa, 1947: 11 (female, male; Brazil).

Material examined. All the specimens were collected as larvae or pupae, reared in laboratory. **ARGENTINA:** MISIONES: Corpus, Puerto Maní: vi.2000, 3 L4, F. Krsticevic leg.; 23.ix.1997, 5 L4, L. Hes leg.; 17.v.2005, 1 larval exuvia, M. Ronderos leg.; ii.2001, 3 ♂♂ 'ex pupa', 1 ♀ 'ex pupa', G. Rossi leg.; Parque Provincial Saltos del Moconá, 27°15'60"S 53°89'16.5"W, 4.xi.2011, 4 ♂♂ 'ex pupa', 1 ♂ pupa 'ex larva', 1 ♂ pupa, 3 L4, E. Lestani leg. **CORRIENTES:** Garapé: 17.vi.1999, 1 pupa, 1 L4, F. Krsticevic leg.; Estación Biológica Corrientes (EBCo), 29.xi.2010, 2 ♂♂ 'ex pupa', 2 ♂♂ 'ex larva', R. Campos leg. **BUENOS AIRES:** City Bell, 6.iii.2001, 1 ♀ 'ex pupa', M. Ronderos leg.

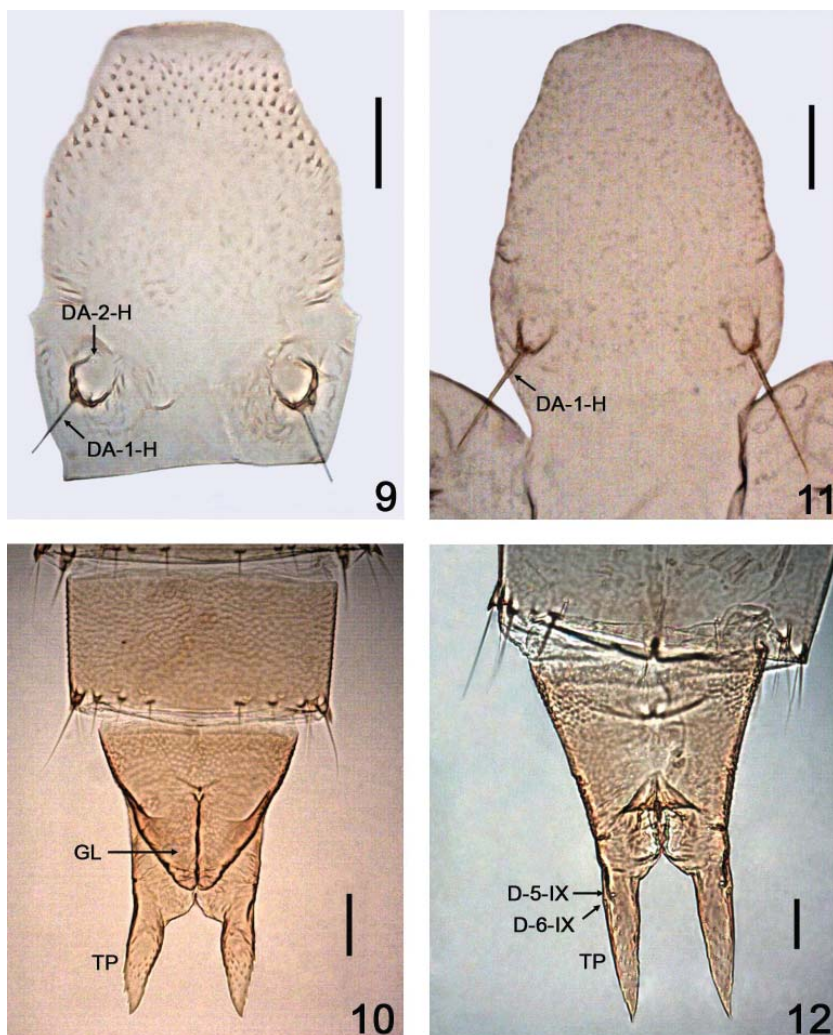
Description. Fourth instar larva (Figs 1–4). Larva length 3.35–5.07 (4.13, $n = 6$) mm. Head capsule (Figs 1–2) pale yellowish, moderately elongate, apex nearly straight, HL 0.150–0.225 (0.193, $n = 12$) mm, HW 0.095–0.170 (0.13, $n = 8$) mm, HR 1.32–1.92 (1.566, $n = 8$), SGW 0.095–0.150 (0.109, $n = 6$), SGR 1.13–1.35 (1.23, $n = 6$). All setae thin, medium-sized to elongate, except setae 'y' stout; chaetotaxy as in Figs 1–2. Antenna stout (Figs 1–2), short. Labrum (Figs 1–2) shorter than greatest basal width, with pair of anterolateral sensilla trichoidea (Figs 1–2); palatum with 2 pairs of contiguous sensilla styloconica, and 2 pairs of sensilla trichoidea underneath (Figs 1–2); messors (Figs 2–3) stout, poorly sclerotized, with 7–8 elongate teeth, five-toothed well developed scopae (Fig. 3). Mandible (Fig. 4) of medium length, stout, hooked, curved, with broad base, 7–8 short subapical teeth, 2 stout, pointed apical teeth, one insertion of setae on ectal margin near hypocondyle; ML 0.047–0.065 (0.06, $n = 9$) mm, MW 0.017–0.020 (0.019, $n = 9$) mm. Maxilla (Figs 1, 2) with short papillae; maxillary palpus (Figs 2, 4) stout, cylindrical, with 6 long apical papillae, two short, lateral papillae, hook-shaped lateral lobe; galeolacinia with long seta. Hypostoma (Figs 1–2) with rounded, mesal, smooth elevation, lateral margin serrate with three stout teeth, the inner one conspicuous. Epipharynx massive, with 2 ventral combs (2 and 4), dorsal comb sclerites enlarged with fringed edge; lateral arms stout, sclerotized, with hyaline lateral curtains; LAW 0.057–0.062 (0.06, $n = 5$) mm, DCW 0.020 ($n = 1$). Hypopharynx elongate, moderately sclerotized, arms nearly straight. Thoracic pigmentation uniformly pale, integument with notorious long setae. Abdominal segments uniformly pale. Caudal segment with 5 pairs of pale, long, thin setae; CSL 0.420–0.490 (0.460, $n = 4$) mm, CSW 0.160–0.176 (0.165, $n = 3$) mm, CSR 2.55–2.75 (2.63, $n = 3$), seta 'o' elongated, OL 0.300–0.400 (0.325, $n = 4$), OD 0.050–0.070 (0.060, $n = 3$) mm.

Male pupa (Figs 5–10, 13–16). Exuviae pale yellowish. Total length 1.72–2.32 (2.07, $n = 6$) mm. Length of cephalothorax 0.79–0.92 (0.85, $n = 6$) mm, width 0.52–0.60 (0.56, $n = 4$) mm. Dorsal apotome (Fig. 9) as long as wide, dorsal margin nearly straight; laterodistal margins of disc surface covered by spinules; tubercle of dorsal apotome sensilla well-developed, base rounded with marginal small spines; DA-1-H elongate, stout, pointed seta, DA-2-H pore; DAL 0.149–0.163 (0.156, $n = 2$) mm; DAW 0.149–0.163 (0.156, $n = 2$) mm; DAW/DAL 1 ($n = 2$). Respiratory organ (Figs 5–6, 13) uniformly yellowish, medium-sized, stout; dorsal surface with short scale-like spines; 5–6 apical pores, 4 laterally on distal half; pedicel length 0.008–0.012 (0.0096, $n = 6$) mm, RO length 0.09–0.11 (0.104, $n = 6$) mm, P/RO 0.08–0.108 (0.09, $n = 6$). Cephalothoracic sensilla as follows: two dorsolateral cephalic sclerite sensilla (Figs 5, 13) on blunt tubercle: DL-1-H long, stout seta, DL-2-H minute seta,



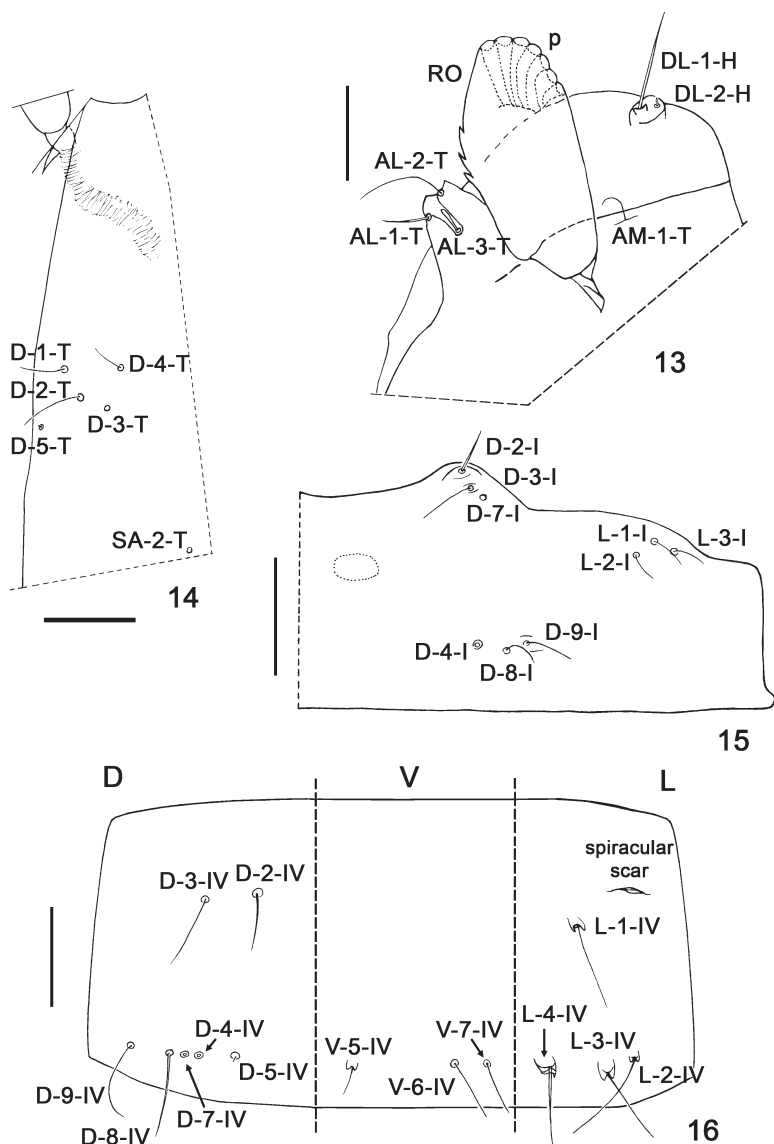
Figs 5–8. *Culicoides bambusicola* Lutz, 1913, male pupa: 5 – cephalothoracic sensilla and respiratory organ; 6 – respiratory organ; 7 – anterolateral sensilla; 8 – clypeal/labral sensillum and ocular sensilla. Scale bars: 0.05 mm. Abbreviations: AL-1-T, AL-2-T, AL-3-T – anterolateral sensilla; AM-1-T – anteromedial sensillum; CL-1-H – clypeal/labral sensillum; DL-1-H, DL-2-H – dorsolateral cephalic sclerite sensilla; O-1-H, O-2-H, O-3-H – ocular sensilla; P – pedicel; p – pore; RO – respiratory organ.

campaniform sensillum not visible; three anterolateral sensilla (Figs 5, 7, 13), AL-1-T medium-sized, stout seta, AL-2-T long, thin seta, AL-3-T campaniform sensillum; anteromedial sensillum (Figs 5, 13) medium sized, thin; dorsal setae (Fig. 14) as follows: D-1-T, D-2-T medium-sized, thin setae, D-3-T campaniform sensillum, D-4-T short seta, D-5-T minute seta, SA-2-T campaniform sensillum. Three ocular (Fig. 8) thin sensilla, O-1-H long seta, O-3-H slightly shorter, O-2-H campaniform sensillum; one clypeal/labral (Fig. 8) medium-sized sensillum. Abdominal segments integument spiculated. First abdominal segment (Fig. 15) with sensilla as follows: D-2-I stout, medium sized seta, D-3-I thin, medium-sized setae, D-7-I pore; L-1-I, L-2-I, L-3-I medium-sized short, thin setae; D-8-I, D-9-I medium-sized, thin setae, D-4-I pore. Fourth segment with setae poorly developed (Fig. 16): 7 dorsal setae, D-2-IV, D-3-IV, long, thin setae, D-2-IV stouter; D-5-IV minute seta, D-4-IV, D-7-IV without



Figs 9–12. *Culicoides bambusicola* Lutz, 1913. 9–10 – male pupa; 11–12 – female pupa; 9, 11 – dorsal apotome; 10, 12 – ninth segment, ventral view. Scale bars: 0.05 mm. Abbreviations: **DA-1-H**, **DA-2-H** – dorsal apotomal sensilla; **GL** – genital lobe; **TP** – terminal process; **D-5-IX**, **D-6-IX** – campaniform sensilla of terminal process.

setae, D-8-IV long, stout seta, D-9-IV long, thin seta; 4 long, lateral setae, L-1-IV, L-2-IV, L-3-IV thin setae, L-4-IV stout seta; 3 ventral setae, V-5-IV short, thin seta, V-6-IV, V-7-IV, medium-sized, thin setae, V-7-IV shorter. Segment 9 (Fig. 10) approximately 1.5× longer than greatest width, length 0.25–0.29 (0.27, $n = 6$) mm, width 0.149–0.186 (0.174, $n = 4$) mm, with posteriorly directed spicules restricted ventrally to basal transverse band; terminal processes stout, short, subparallel with pointed tip; surface covered by sparse spicules, length 0.09–0.10 (0.093, $n = 6$) mm.



Figs 13–16. *Culicoides bambusicola* Lutz, 1913, male pupa: 13 – cephalothoracic sensilla and respiratory organ; 14 – dorsal setae; 15 – first abdominal segment chaetotaxy; 16 – fourth abdominal segment chaetotaxy. Scale bars: 0.05 mm. Abbreviations: AL-1-T, AL-2-T, AL-3-T – anterolateral sensilla; AM-1-T – anteromedial sensillum; DL-1-H, DL-2-H – dorsolateral cephalic sclerite sensilla; p – pores; RO – respiratory organ; D-1-T, D-2-T, D-3-T, D-4-T, D-5-T – dorsals; SA-2-T – supraalar; D-2-I, D-3-I, D-4-I, D-7-I, D-8-I, D-9-I – dorsal sensilla of first abdominal segment; L-1-I, L-2-I, L-3-I – lateral sensilla of first abdominal segment; D-2-IV, D-3-IV, D-4-IV, D-5-IV, D-7-IV, D-8-IV, D-9-IV – dorsal sensilla of fourth abdominal segment; L-1-IV, L-2-IV, L-3-IV, L-4-IV – lateral sensilla of fourth abdominal segment; V-5-IV, V-6-IV, V-7-IV – ventral sensilla of fourth abdominal segment.

Female pupa (Figs 11–12). Similar to male with following sexual differences. Total length 2.54–2.88 (2.71, $n = 2$) mm. Dorsal apotome (Fig. 11) DAL 0.162–0.200 (0.181, $n = 2$) mm; DAW 0.174–0.200 (0.187, $n = 2$) mm; DAW/DAL 1.00–1.07 (1.035, $n = 2$). Respiratory organ length 0.122–0.140 (0.131, $n = 2$) mm; width 0.048–0.062 (0.055, $n = 2$) mm; pedicel length 0.010 ($n = 2$) mm; P/RO 0.071–0.082 (0.077, $n = 2$). Cephalothorax length 1.02–1.10 (1.06, $n = 2$) mm, width 0.74–0.80 (0.77, $n = 2$) mm. Segment 9 (Fig. 12) with posteriorly directed spicules restricted to narrow anterior band, not connected to central inverted V-shaped patch of spicules; mesal spicules patch not extending to terminal processes, these moderately elongated, subparallel with pointed, dark tips; ventral surface of terminal processes with spicules present on each side of midline; length 0.272–0.320 (0.296, $n = 2$) mm, width 0.184–0.240 (0.212, $n = 2$) mm; terminal processes length 0.09–0.11 (0.10, $n = 2$) mm.

Taxonomic notes. RONDEROS & SPINELLI (2000) in their last redescription of *Culicoides bambusicola* omitted the description of the palatum, maxilla and hypopharynx of the fourth instar larva, as well as of the cephalothoracic sensilla of the pupa. The fourth abdominal segment of the pupae was incompletely described and its sensilla were erroneously described.

In the genus *Culicoides* there are three sensilla on the dorsolateral sclerite: 2 setae (generally one larger, one smaller) and a campaniform sensillum (Borkent, pers. comm.). For the species with 2 setae such as *C. bambusicola*, there should probably be also a separate campaniform sensillum, which could not be observed in the studied material.

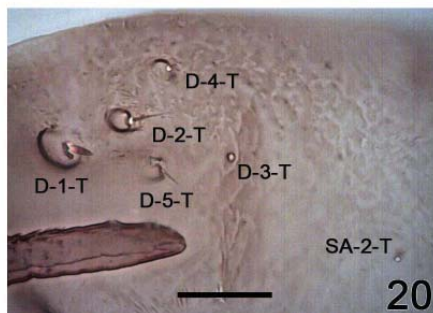
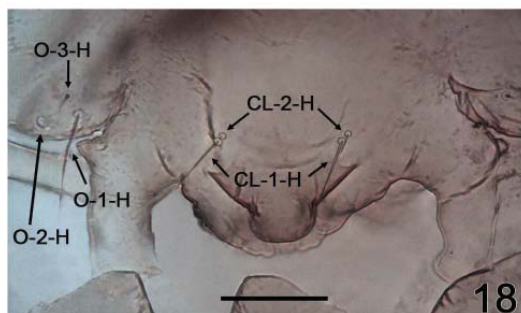
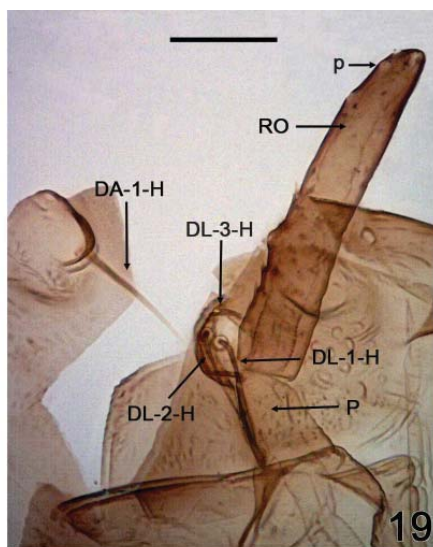
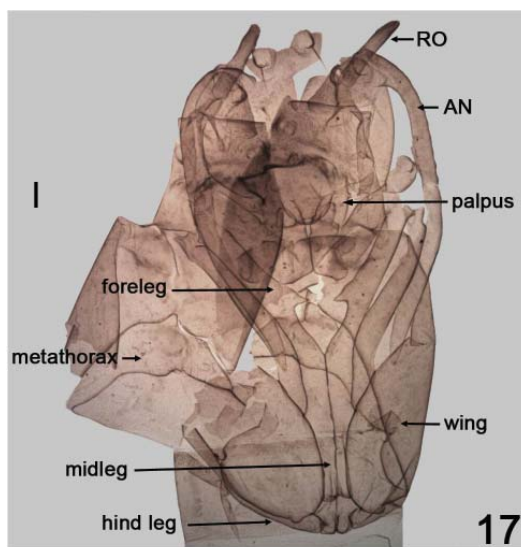
The subgenus *Cotocripus* Brèthes, 1912 (BORKENT & SPINELLI 2007, SPINELLI et al. 2009) is represented by six species, of which only the immatures of *C. bambusicola* have been studied. Therefore, we compared it with *C. debilipalpis*, which is included in the subgenus *Haematomyidium* Goeldi, 1905 and develops in clean water of tree holes. The immatures of both species are very similar; the few differences between the fourth instar larvae are the following: *C. debilipalpis* has the antenna less conspicuous, the head ventral seta 'y' is stouter and mandible has two elongated, small pointed teeth, the inner tooth is smaller. The pupa of *C. debilipalpis* has the respiratory organ longer with large scale-like spines; the dorsal apotome has the disc surface completely covered with spines.

Distribution. Colombia, Brazil and Argentina (Misiones, Corrientes and Buenos Aires provinces).

Culicoides insignis Lutz, 1913

(Figs 17–28)

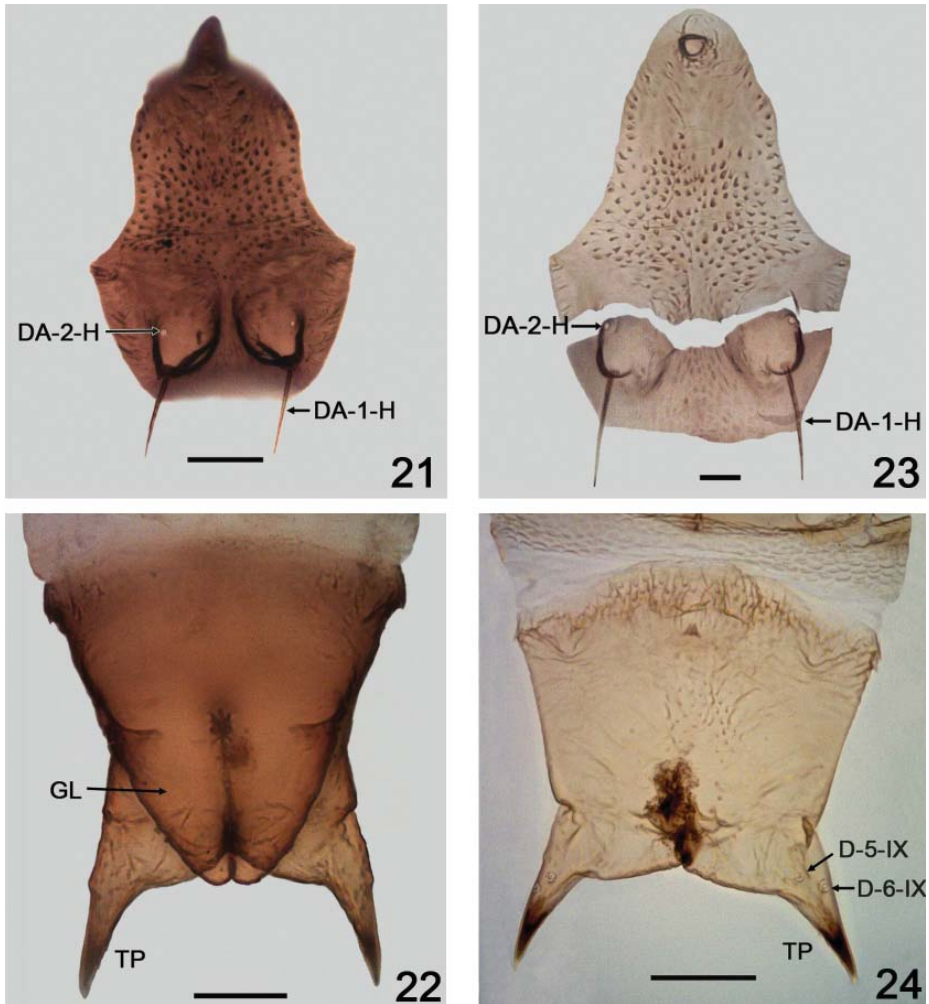
Culicoides insignis Lutz, 1913: 50 (male, female, pupa, fig. wing; Brazil); COSTA LIMA (1937: 415) (fig. palpus; Brazil); FLOCH & ABONNENC (1942: 1) (fig. wing, palpus; French Guiana); BARBOSA (1947: 20) (fig. male genitalia); FOX (1948: 25) (notes on female); BARBOSA (1952: 16) (notes on Lutz collection); WIRTH & BLANTON (1956: 319) (redescr., male lectotype design., distrib.); FORATTINI et al. (1956: 195) (larva, pupa; Brazil); FORATTINI (1957: 223) (redescr., distrib.); WIRTH & BLANTON (1959: 285) (redescr., figs; Panama); WILLIAMS (1964: 463) (habitat larva; Trinidad); LINLEY (1965: 57) (pupa; Jamaica); CAVALIERI & CHIOSSONE (1966: 148) (Argentina); WIRTH (1974: 25) (catalog, species south of USA); WIRTH & BLANTON (1974: 57) (redescr.; Antilles); AITKEN et al. (1975: 130) (Trinidad); BLANTON & WIRTH (1979: 106) (female, male genitalia, pupa, figs.; Florida records); SPINELLI & WIRTH (1985: 52) (in key, wing photo); WIRTH et al. (1988: 16) (Neotropical atlas, wing); SPINELLI & RONDEROS (1991: 86) (Uruguay); SPINELLI & WIRTH (1993: 34) (list of Argentinean species); SPINELLI et al. (1993: 53) (in key, wing photo, diagnosis, pupa); BORKENT & WIRTH (1997: 71) (world catalog); SPINELLI (1998: 325) (list of Argentinean species); RONDEROS & SPINELLI (1998: 79) (key); BORKENT & SPINELLI (2000: 34) (catalog, species



Figs 17–20. *Culicoides insignis* Lutz, 1913. 18 – male pupa; 17, 19–20 – female pupa; 17 – cephalothorax, ventral view; 18 – clypeal/labral sensilla and ocular sensilla; 19 – dorsal apotomal sensillum, cephalothoracic sensilla and respiratory organ; 20 – dorsal setae. Scale bars: 0.05 mm. Abbreviations: AN – antennae; CL-1-H, CL-2-H – clypeal/labral sensilla; DA-1-H – dorsal apotomal sensillum; DL-1-H, DL-2-H, DL-3-H – dorsolateral cephalic sclerite sensilla; D-1-T, D-2-T, D-3-T, D-4-T, D-5-T – dorsal setae; O-1-H, O-2-H, O-3-H – ocular sensilla; P – pedicel; p – pores; RO – respiratory organ; SA-2-T – supraalar.

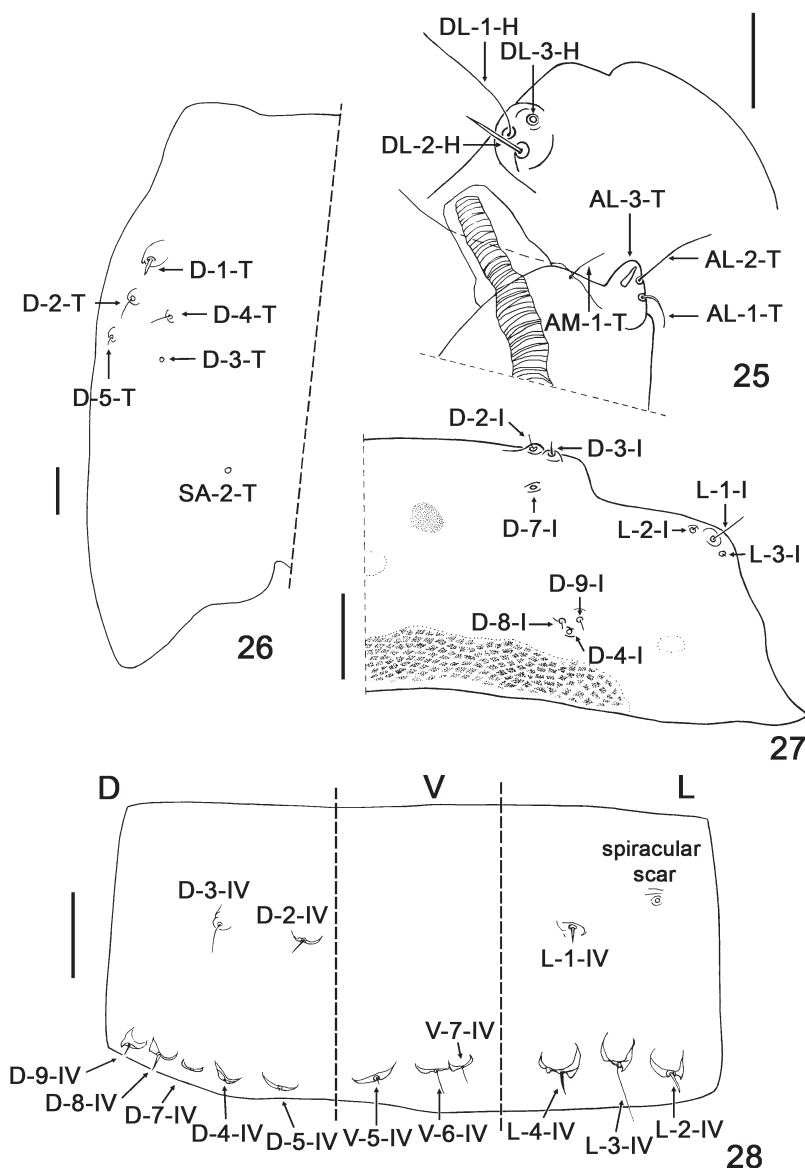
south of USA); SPINELLI et al. (2005: 139, 142) (key, wing photo; Argentina); SPINELLI & RONDEROS (2005: 63) (wing); BORKENT & SPINELLI (2007: 68) (Neotropical catalog); BORKENT & GROGAN (2009: 14) (Nearctic catalog); RONDEROS et al. (2011: 1188) (list of Argentinian species); BORKENT (2013: 86) (online catalog).
Culicoides inamollae Fox & Hoffman, 1944: 110 (male, female; Puerto Rico).
Culicoides painteri Fox, 1946: 257 (female; Honduras).

Specimens examined. All the specimens were collected as pupa, reared in laboratory. **ARGENTINA:** CORRIENTES: arroyo Ambrosio, 15.ix.2010, 28°15'16.4"S 58°50'33.6"W, 44 m, 1 ♂ 'ex pupa', F. Díaz leg. **ENTRE RÍOS:** Santa Ana, 23.ix.1984, 1 ♂, 1 ♀ 'ex pupa', Balseiro-Spinelli leg. **BUENOS AIRES:** Magdalena, arroyo Zapata, 15.xii.1981, 1 ♀ 'ex pupa', G. Spinelli leg.



Figs 21–24. *Culicoides insignis* Lutz, 1913. 21–22 – male pupa; 23–24 – female pupa; 21, 23 – dorsal apotome; 22, 24 – ninth segment, ventral view. Scale bars: 0.05 mm. Abbreviations: **DA-1-H**, **DA-2-H** – dorsal apotomal sensilla; **GL** – genital lobe; **TP** – terminal process; **D-5-IX**, **D-6-IX** – campaniform sensilla of terminal process.

Description. Male pupa (Figs 18, 21–22, 25–28). Exuvia brown. Total length 2.82 mm. Cephalothorax length 1.073 mm. Dorsal apotome (Fig. 21) dark brown, slightly wider than long, distal margin rounded, bearing a short pointed projection; disc surface covered with spinules; tubercle of dorsal apotome sensilla well-developed, with quadrangular base; DA-1-H medium-sized, stout, pointed seta of 0.037 mm, DA-2-H pore; dorsal margin nearly straight, posterior half surface wrinkled; DAL 0.156 mm; DAW 0.182 mm; DAW/DAL 1.17. Respiratory organ elongated, uniformly brown, basal half surface with short scale-like spines; 6 apical pores; pedicel stout, pedicel length 0.074 mm, RO length 0.186 mm, P/RO 0.40.



Figs 25–28. *Culicoides insignis* Lutz, 1913, male pupa: 25 – cephalothoracic sensilla; 26 – dorsal setae; 27 – first abdominal segment chaetotaxy; 28 – fourth abdominal segment chaetotaxy. Scale bars: 0.05 mm. Abbreviations: AL-1-T, AL-2-T, AL-3-T – anterolateral sensilla; AM-1-T – anteromedial sensillum; DL-1-H, DL-2-H, DL-3-H – dorsolateral cephalic sclerite sensilla; D-1-T, D-2-T, D-3-T, D-4-T, D-5-T – dorsal setae; SA-2-T – supraalar; D-2-I, D-3-I, D-4-I, D-7-I, D-8-I, D-9-I – dorsal sensilla of first abdominal segment; L-1-I, L-2-I, L-3-I – lateral sensilla of first abdominal segment; D-2-IV, D-3-IV, D-4-IV, D-5-IV, D-7-IV, D-8-IV, D-9-IV – dorsal sensilla of fourth abdominal segment; L-1-IV, L-2-IV, L-3-IV, L-4-IV – lateral sensilla of fourth abdominal segment; V-5-IV, V-6-IV, V-7-IV – ventral sensilla of fourth abdominal segment.

Cephalothoracic sensilla as follows: dorsal cephalic sclerite sensilla (Fig. 25) conspicuous, with three sensilla, DL-1-H long, thin seta, DL-2-H medium-sized seta, stout, DL-3-H campaniform sensillum; anterolateral sensilla (Fig. 25) moderately developed, with three sensilla, AL-1-T short, thin seta, AL-2-T medium-sized, thin seta, AL-3-T campaniform sensillum; anteromedial sensillum (Fig. 25) short, thin; dorsal setae (Fig. 26) as follows: D-1-T spur, D-2-T, D-3-T campaniform sensillum, D-4-T thin, medium-sized, D-5-T short, SA-2-T campaniform sensillum. Three ocular sensilla (Fig. 18), O-1-H stout, long seta, O-3-H thin, short seta, O-2-H campaniform sensillum; two clypeal/labral thin sensilla (Fig. 18), CL-1-H long seta, CL-2-H short seta. Abdominal segments integument spiculated. First abdominal segment (Figs 27) with setae as follows: D-2-I and D-3-I short, thin setae, D-7-I pore; L-1-I and L-3-I minute seta, L-2-I medium-sized, stout seta; D-8-I, D-9-I short, thin setae, D-4-I pore. Fourth segment with all tubercles with small and flattened base (Fig. 28): 7 dorsal setae: D-2-IV spur-like seta, D-3-IV, medium-sized, thin seta; D-5-IV minute seta, D-4-IV, D-7-IV without setae, D-8-IV, D-9-IV short setae, D-8-IV stouter; 4 lateral setae, L-1-IV, L-2-IV, L-4-IV spur-like setae, L-3-IV long, thin seta; 3 ventral setae: V-5-IV minute seta, V-6-IV, V-7-IV short, thin setae. Segment 9 (Fig. 22) approximately $1.25\times$ longer than greatest width, length 0.234 mm, width 0.186 mm, with posteriorly directed spicules restricted dorsally to mesal area; terminal processes triangular, short, subparallel with pointed, dark tips; length 0.092 mm; surface covered by sparse spicules.

Female pupa (Figs 17, 19–20, 23–24). Similar to male with following sexual differences. Total length 2.56 mm. Dorsal apotome (Fig. 23) DAL 0.149 mm; DAW 0.158 mm; DAW/DAL 1.06. Respiratory organ (Figs 17, 19) length 0.17–0.19 (0.18; $n = 2$) mm; width 0.03–0.042 (0.036, $n = 2$) mm; pedicel length 0.034–0.036 (0.035; $n = 2$); P/RO 0.185–0.201 (0.193; $n = 2$). Cephalothorax (Fig. 17) length 1.10 mm, width 0.56 mm. Dorsal setae as in Fig. 20. Segment 9 (Fig. 24) with dorsomesal spicule patch extending to terminal processes; length 0.204–0.216 (0.21; $n = 2$) mm, width 0.175–0.200 (0.187; $n = 2$) mm; terminal processes length 0.060–0.062 (0.061; $n = 2$) mm.

Taxonomic notes. Of the 39 Neotropical species of *Culicoides* in the subgenus *Hoffmania* Fox, 1948, only five have been described as pupae: *C. annettae* Spinelli & Borkent, 2004, *C. charruus* Spinelli & Martinez, 1992, *C. hylas* Macfie, 1940, *C. insignis* Lutz, 1913 and *C. maruim* Lutz, 1913.

The pupae of those species are very similar. The main differences observed between them are the following: the pupa of *C. charruus* which was fully described (RONDEROS et al. 2008) has the dorsal apotome shorter without a mesal projection; the respiratory organ is slightly broader at apex and truncate, with 12–16 apical pores; the anterior ocular sensilla (O-3-H) are longer; the clypeal/labral sensilla are one minute seta, the other a pore; the D-5-IV sensilla of fourth abdominal segment is long and thin and the terminal processes of segment 9 are longer. The pupa of *C. annettae* (SPINELLI & BORKENT 2004) has more spinose respiratory organ and the pores are distributed in a row along its apical half. The pupa of *C. maruim* which was poorly described (SPINELLI et al. 1993) is pale yellowish and its respiratory organ has 7 apical pores. The description of the pupa of *C. hylas* (FORATTINI 1957) is incomplete, nevertheless it shows that the respiratory organ has 17 apical pores and the tips of the terminal processes are not dark.

Distribution. USA, Mexico, Central America and Caribbean to eastern Argentina (Corrientes, Entre Rios, and Buenos Aires provinces).

Discussion

FOX (1942) studied the correlation between some morphological structures of the pupae of *Culicoides* and suggested that variation in the respiratory organ and the ninth segments are related to environment. The presence of scale-like spines in the respiratory organ of the pupa is typical for species breeding in tree holes (RONDEROS et al. 2010). This character state was observed also in species breeding in similar environments with clean water, as also occurs with *C. bambusicola* whose pupae were found in bamboo canes. On the other hand, the respiratory organ of the pupa of *C. insignis* is longer and it bears less scale-like spines on basal half.

The terminal processes of the nine segments of *C. insignis* are shorter and more pointed than in *C. bambusicola*. These notorious differences between both species could be due to the different environments where they develop.

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